Scientific Papers. Series B, Horticulture. Vol. LXVII, No. 1, 2023
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRELIMINARY EVALUATION ON GETTING SPROUTS IN SORREL ON DIFFERENT SUBSTRATES

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Abstract

In recent years, there is an increasing trend worldwide in consuming microplants (microgreens / sprouts). Due to their benefits for human health, less growing requirements, high nutritional composition and their versatility in various cuisines, microplants become of great interest. Therefore, there is a need in finding sustainable possibilities for obtaining such sources, rich in health-promoting compounds. Thereby, the objective of this paper is to present the results of an experimental design that involves the obtaining of Rumex acetosa L. (sorrel) sprouts (microplants) on different cultivation substrates. The biological material consisted of seeds of sorrel, and six different substrates varying the ratio between perlite, banana peel and agar. The experiments were performed in a non-aseptic system. After 14 days, the seed germination capacity and the number of leaves were recorded. Also, several physicochemical and biochemical analyses performed for the resulting sprouts (microplants), such as micro and macronutrients content, assimilatory pigments and dry matter (DM). The most promising cultivation substrates for sorrel sprouts (microplants) are perlite and the mixtures of perlite and agar. In terms of sprouts quality, both variant 2 (perlite 75% with agar 25%) and variant 4 (perlite 25% with agar 75%) of cultivation were highlighted.

***Key words**: cultivation substrates, germination, nutrients, quality of sprouts (microplants), sorrel.*

INTRODUCTION

In recent years, with the increased interest in healthy eating, society has turned its attention to fresh or functional foods, such as small-scale vegetables microplants, called “vegetable confetti” (Bhaswant et al., 2023). Microplants, both sprouts and microgreens, have higher nutritional value than mature vegetables or seeds (e.g., vitamins, chlorophyll, macro and microelements), reveal exciting flavours and tastes, and can be quickly cultivated under the influence of LEDs artificial light, being more and more diversified and more often found on supermarket shelfs (Zhang et al., 2021).

One of the plants that claim to be produced as sprouts is sorrel (*Rumex acetosa* L.) (Choe et al., 2018). Sorrel is a green plant with spontaneous growth that belongs to the *Polygonaceae* family (Li et al., 2022). This is a fast-growing perennial plant, resistant to various abiotic factors (e.g., low temperatures in winter) and biotic (diseases or pests). The leaves or stems of *Rumex* are harvested for fresh consumption and used as raw in salads or for various culinary preparations, bringing a nutritional contribution to a balanced diet. (Spínola et al., 2018; Barbu et al., 2021). Unfortunately, it has a high oxalic acid content, which can harm health if ingested in large doses, affecting the kidneys and renal tubule (Farré et al., 1989). However, sorrel eaten in moderation prevents and controls some diseases. Seeds, leaves, stems, or roots are used worldwide as "drugs" in alternative medicine (Bello et al., 2019). As a medicinal plant, sorrel (*Rumex acetosa* L.) can treat gastrointestinal disorders and skin diseases, improve eyesight, stabilize blood pressure, and prevent scurvy (Barbu et al., 2021). In addition to the nutritional properties, sprouts involve minimal resources such as limited space, various nutrient substrates (e.g., peat, coconut coir, composted organic wastes, vermiculite, perlite), limited water, and zero use of pesticides (Pascual et al., 2018).

The aim of the present study was to assess the preliminary results regarding non-aseptic system for sorrel sprouts (microplants) production in controlled conditions, under LEDs light. The method is an ecological and economical option because it uses organic residues (banana peels), with an essential nutritional supply for the sprouts (sprouts). The obtained sorrel sprouts (microplants) were quantitatively and qualitatively analysed in order to highlight the most appropriate cultivation substrate for this production system.

MATERIALS AND METHODS

Biological material

The biological material consisted of sorrel (*Rumex acetosa* L.) organic certified seeds (ecological and untreated) from the commercial company Italian Sprout Srl, Italy.

The experimental design

In order to obtain the sprouts (microplants), sorrel seeds were grown *in vitro*. The experiments were carried out on five types of cultivation substrates, which combined several solid components such as perlite, agar, and banana peels with distilled water, as liquid component (Table 1).

Each experimental variant was performed in 3 replicates, with 10 glass containers each.

The seeds were sown in glass containers and placed in a cultivation chamber at 21°C ± 2°C temperature, in non-aseptic conditions and exposed to 16/8 h photoperiod artificial LEDs lights (Lee et al., 2014, Enache & Livadariu, 2016) for 13 days at an average luminous flux of 1140 lm.

Table 1. Experimental variants used in the study

Experimental variants	Composition of the nutrient substrate
V1 (CONTROL)	perlite 100%
V2	perlite 75% agar 25%
V3	perlite 50% agar 50%
V4	perlite 25% agar 75%
V5	perlite 75% organic banana peels 25%
V6	perlite 50% organic banana peels 50%
V7	perlite 25% organic banana peels 75%

Methods of analysis

1. Seed germination capacity

The germination capacity (GC%) was determined by counting the number of germinated seeds in glass containers *per* experimental variants. The results are calculated in percentages using the formula developed by Fredrick et al. (2015):

$$GC(\%) = \frac{n_g}{n_t} \times 100$$

n_g = the number of germinated seeds,
n_t = the total number of seeds

2. The number of leaves

Morphometric measurements of the sprouts consisted of counting the number of leaves per microplant using EPSON Model expression 11000XL scanner which uses the image analyser software WinFOLIATM. The results represent the average number of leaves.

3. Dry matter content (DM)

The dry matter (DM) content was determined according to the European Pharmacopoeia 7th edition methods (2010).

4. Assimilatory pigments

Assimilatory pigments content was performed using an adapted method of Wellburn (1994).

The obtained results were expressed as mg chlorophyll a (Chl a), b (Chl b), and total chlorophyll (Chl T) per g fresh weight (mg/g FW).

5. Micro and macronutrients analysis

The fresh sorrel sprouts samples were grinded and three samples were subjected to microwave extraction, as follows: 0.250 g, 8 ml HNO_3 and 2 ml H_2O_2. The control consisted of 8 ml HNO_3 and 2 ml H_2O_2. The samples were subjected to

ICP-MS analysis Agilent 7700X (Bashdar & Rasul, 2023). The analysis was realised based on the calibration curve of the multi-element standard Agilent Technologies solution.
The results of the quantitative determinations are presented as the average of at least 3 determinations accompanied by the standard deviation.
The comparison between the experimental variants was evaluated by one-way ANOVA.

RESULTS AND DISCUSSIONS

Preliminary results regarding sorrel sprouts (microplants) production, on different growing substrates based on perlite, agar and banana peels, in non-aseptic system (environment), are presented as follows.

1. Results on GC

The effect of the cultivation substrate on the germination capacity of sorrel seeds is presented in Table 2.

Table 2. Germination capacity values

Experimental variants	GC % ± SD
V1	89.33 ± 3.05 [a]
V2	86.67 ± 1.52 [ab]
V3	84.00 ± 2.00 [ab]
V4	83.33 ± 1.52 [b]
V5	5.33 ± 1.15 [c]
V6	n.a*
V7	n.a*

*n.a.= not available. Different letters indicate significant difference among the experimental variants.

The germination capacity of sorrel seeds was analysed for only five experimental variants (V1 to V5). Due to the massive microbiological contamination in the other two experimental variants, V6 and V7, the seed germination capacity could not be determined.
Comparing the collected data with those from the control, the best GC% was obtained when sorrel was germinated on perlite. No significant differences were seen between the control (V1) and V2 and V3 experimental variants. However, lowest values regarding GC% were registered in V5 variant, were sorrel was seeded in a substrate mixture of perlite 75% and organic banana peels 25%.
These results are similar to those obtained by Fani et al. (2012), testing the germination capacity of sheep sorrel (*Rumex acetosella* L.) in Petri dishes on soaked filter paper with solutions of different concentrations of gibberellic acid at 25/15°C (day/night) after four weeks.

2. Results on the number of leaves

The effect of substrate cultivation on the growth of sorrel sprouts in terms of the number of leaves is presented in Table 3.

Table 3. The number of leaves

Experimental variants	No. of leaves ± SD
V1	1.67±0.36
V2	1.47±0.53
V3	1.40±0.52
V4	1.57±0.60
V5	n.a*
V6	n.a*
V7	n.a*

*n.a.= not available

Although the germination percentage results were recorded for V1 to V5 variants, the number of leaves indicator was evaluated only for the V1-V4 variants, as in the case of V5 variant the sprouts showed reduced growth. Moreover, the presence of banana peels in the substrate, negatively influence microplants growth, most probably due to the high microbial contamination of this intensive nourishing non-aseptic substrate.
Although some variations within the number of leaves were seen among the analysed experimental variants, statistically, there are no significant differences.

3. Results on dry matter content

Was performed for four experimental variants (Figure 1).

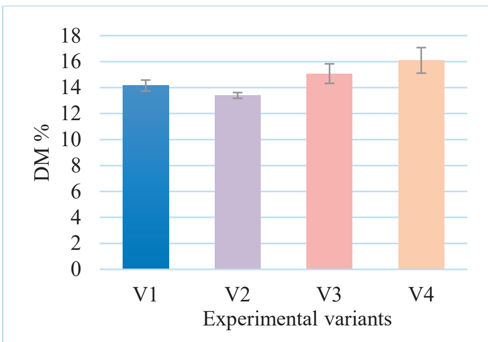

Figure 1. Dry matter content

Among these, the highest value was registered by the experimental variant V4 (16.10%), having as solid substrate perlite 75% and agar 25%. These results were significantly different from the control variant V1 (14.16%), were the solid substrate was based on perlite 100%. On the other hand, the lowest value was registered by the V2 (13.40%) variant, having as substrate two solid components, perlite 75% and agar 25%.

4. Results on the photosynthetic pigments

The effect of cultivation substrate on the accumulation of photosynthetic pigments of sorrel sprouts are presented in Figures 2, 3, and 4. The sorrel sprouts accumulated the highest photosynthetic pigments in the case of control variant with a value of 13.41 mg/g FW Chlorophyll a, while V4 having with 1.04 mg/g FW less (Figure 2).

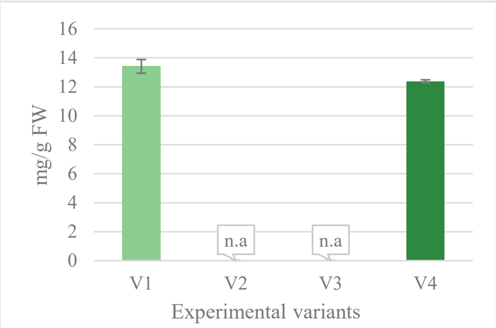

Figure 2. Total content of photosynthetic pigments - Clorophyll a

Comparative to the control, for V4 variant Chlorophyll b accumulation was with 1.03 mg/g FW less than of 11.82 mg/g FW in V1(Figure 3).

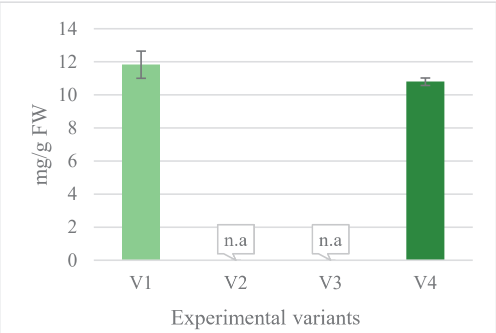

Figure 3. Total content of photosynthetic pigments - Clorophyll b

With regard to the total chlorophyll content, can be observed also an increase with 2.07 mg/g FW for control variant then V4 (23.16 mg/g FW) (Figure 4).

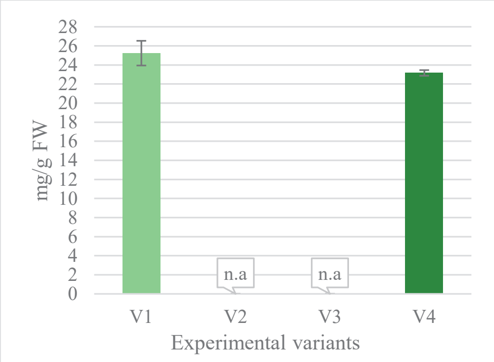

Figure 4. Total content of photosynthetic pigments

Our results are similar to those obtained by Kowitcharoen et al. (2021), who analyzed the total chlorophyll content in selected culinary microgreens that belong to *Brassicaceae, Fabaceae, Pedaliaceae, Polygonaceae Convolvulaceae* and *Malvaceae* families. The result registered for *Fagopyrum esculentum* Moench was of 37.85 mg/100 g, for *Pisum sativum* L. of 12.35 mg/100 g and for *Lens culinaris* Medicus of 112.62 mg/100 g.

5. Results on the micro and macronutrients

The micronutrients Mn, Cu, and Zn were determined and expressed as mg/Kg FW, while the macronutrients Na, Mg, P, K, Ca as mg/ 100 g FW.

The determination of Na content was performed for V1-V4 experimental variants. Among these, the highest value of the Na was registered by the experimental variant V2 (121.45 mg/100 g FW) compared to the control variant V1(113.80 mg/100 g FW). On the other hand, the lowest value was registered by the V4 variant, having 55.26 mg/100 g FW. The results are presented in Figure 5.

The determination of Mg showed that the highest value was registered by the experimental variant V2 with 41.34 mg/100 g FW comparative to the control variant V1 having 36.83 mg/100 g FW. On the other hand, the lowest value was registered by the V3 variant (33.33 mg/100 g FW). The results are presented in Figure 6.

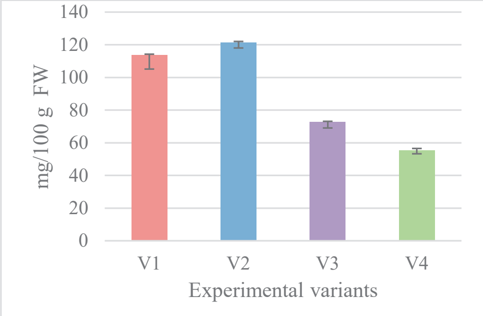

Figure 5. Sodium content in sorrel sprouts

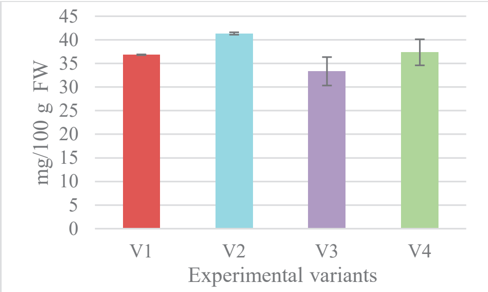

Figure 6. Magnesium content in sorrel sprouts

The determination of total phosphorus content was performed and the highest value was registered by the control experimental variant V1 (100.28 mg/100 g FW). Between V2-V4 variants a value of 94.287 mg/100 g FW was registered in the case of V4. On the other hand, the lowest value was registered by the V3 having as a nutrient substrate a solid component - perlite (50%) and agar (50%) with a value of 82.87 mg/100 g FW. For P the control variant has the highest values, so we can say that adding the agar to perlite lowers the P accumulation in sorrel, the best cultivation variant being 100% perlite. The results are presented in Figure 7.

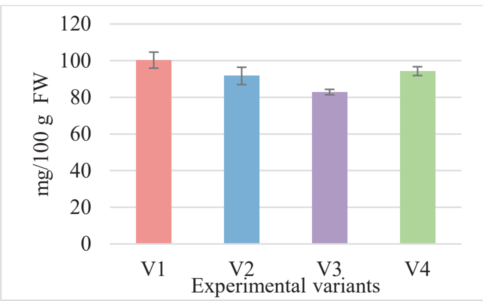

Figure 7. Phosphorus content in sorrel sprouts

The determination of total potassium content showed the highest value in V2, having 158.22 mg/100 g FW, while the control variant V1 123.80 mg/100 g FW. At the opposite pole, the lowest value was registered by the V4 with 143.78 mg/100 g FW. The results are presented in Figure 8.

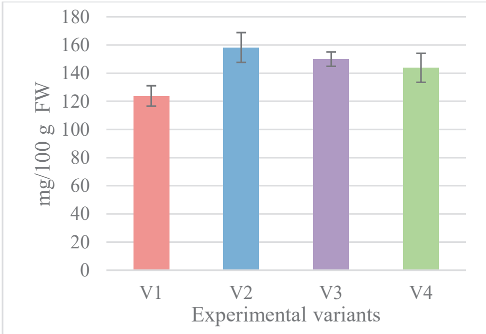

Figure 8. Potassium content in sorrel sprouts

The highest value of the Ca was registered by the experimental variant V4 (45.58 mg/100 g FW), variant having as a nutrient substrate a solid components perlite (25%) and agar (75%), compared to the control variant V1 (25.03 mg/100 g FW). On the other hand, the lowest value was registered by the V3 (33.82 mg/100 g FW) having as a nutrient substrate a solid components perlite (50%) and agar (50%). The results are presented in Figure 9.

For all the experimental variants, the content of calcium and potassium was higher than that of the control variant.

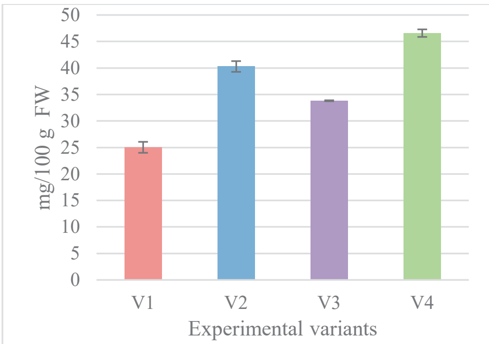

Figure 9. Calcium content in sorrel sprouts

Also, manganese content was performed for all experimental variants. Among these, the highest value of the manganese was registered in the case of V2 having a value of 17.95 mg/kg FW, comparative to the control variant V1 having 14.49 mg/kg FW. The lowest value was registered in the case of V3 having 14.41 mg/kg FW. The results are presented in Figure 10.

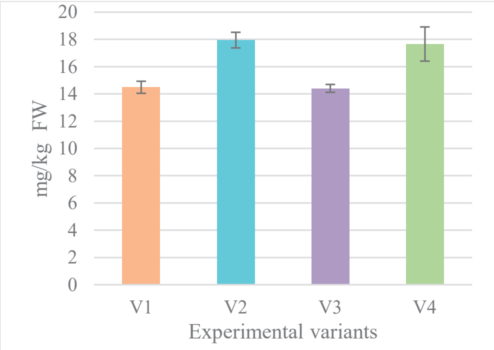

Figure 10. Manganese content in sorrel sprouts

Within micro nutrients, also copper content was quantified. Among all variants, the highest value of the copper was registered in experimental variant V4, recording 3.12 mg/kg FW, comparative to the control variant V1 having 0.61 mg/100 g FW. On the other hand, the lowest value was registered by the V2 having 0.5 mg/kg FW.

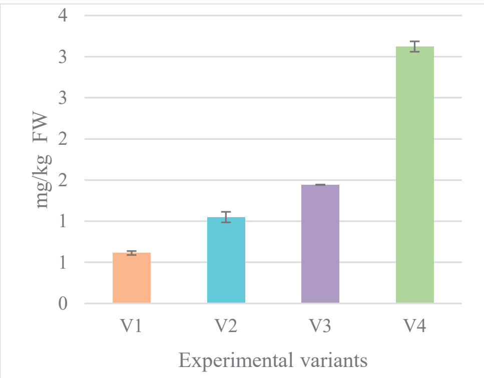

Figure 11. Copper content in sorrel sprouts

Continuing on the quantification of micro nutrients, zinc content was evaluated for all experimental variants. Among these, the highest value of the zinc was registered by the experimental variant V4, having as a substrate two solid components (perlite 50% and organic banana peels 50%) with a value of 7.82 mg/kg FW, greater than the control variant V1 having 6.74 mg/kg FW. Between variants, the lowest value was registered by the V2 having 6.82 mg/kg FW.

For all the experimental variants, the content of zinc and copper was higher than that of the control variant.

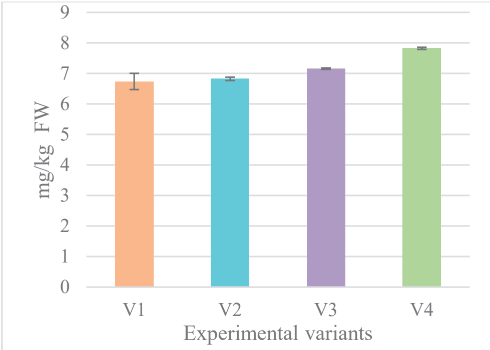

Figure 12. Zinc content sorrel sprouts

We can compare the results obtained for the qualitative analysis of sorrel sprouts regarding the content of macro and micronutrients with those obtained by Idris et al. (2011), who analysed dry leaves of mature plants of *Rumex acetosa* L., as follows: Na (28.61±0.48 mg/ 100 g DM), Mg (73.56±0.02 mg/100 g DM), P (7.73±0.07 mg/100g DM), K (2132.85±3.52 mg/100 g DM), Ca (53.25±0.05 mg/100 g DM), Mn (13.59±0.04 mg/100 g DM), Cu (0.85±0.04 mg/100 g DM), and Zn (2.66±0.01 mg/100 g DM).

According to these results, the recorded values demonstrate that the V2 variant with perlite 75% and agar 25%, had the highest content of macronutrients such as: Na (121.45 ± 3.42 mg/100 g FW), Mg (41.34 ±0.25 mg/100 g FW), K (158.22±10.59 mg/100 g FW), Ca (40.28 ± 1.02 mg/100 g FW) and Mn micronutrient (17.95 ±0.57 mg/kg FW).

In the V4 variant (perlite 25% and agar 75%) sorrel sprouts accumulated the highest Ca (46.58±0.714 mg/100 g FW), but for P content (94.29 ± 2.41 mg/100 g FW) the value is lower than the V1 control variant, but higher than V2 and V3. For micronutrients we can highlight the V4 results obtained in sorrel sprouts for Cu (3.12±0.06 mg/kg FW) and Zn (7.82± 0.04 mg/kg FW).

CONCLUSIONS

Comparing different cultivation substrates reveals that banana peels, in a non-aseptic system, is not suitable for sprouts (microplants) production. This fact banana peels are strongly related to the massive microbial contamination of V5, V6, V7 experimental variants.

The determination of total dry matter content of sorrel sprouts (microplants) indicates that V4 variant is optimal for this parameter.

The experimental variant V2 composed of perlite 75% and agar 25% registered significant values regarding the number of germinated seeds and the content of macro and micronutrients as Na, Mg, K and Mn.

Sorrel grown on V_4 variant based on perlite 25% and agar 75%, presented a high content of chlorophyll and macro and micronutrients as Ca, P, Cu and Zn.

Adding agar to the perlite cultivation substrate, lead to a decrease accumulation of P and assimilatory pigments content in sorrel sprouts (microplants).

It is essential to highlight the fact that the results indicate the influence of substrate cultivation on the biochemical content of sorrel sprouts (microplants). Considering these aspects, the preliminary results offer a perspective of sorrel sprout (microplants) production, thereby using the cultivation substrate associated with the accumulation of the health-promoting compound.

ACKNOWLEDGEMENTS

This research was funded by the University of Agronomic Sciences and Veterinary Medicine of Bucharest through the Project no. 1067/15.06.2022, *Production technology of aromatic microplants in an innovative cultivation system.*

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